Variations in Tissue Development and Secondary Product Elaboration of *Hedychium coronarium* J. König Floral Cultures Grown on Different Media

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Abstract

The studies on the variations in tissue development and secondary productelaboration of *Hedychium coronarium* J. König, locally known as camia, in culture on different growth media, using the floral tube part, are reported.

Introduction

The use of biotechnology in the study and harvest of important natural products is a valued approach especially now that the natural resources are fast dwindling. Fascination for plant essential oils, for instance, dates back to antiquity and their production in plant tissue cultures has long been sought for. However, experience shows that not all natural products produced by the plant could be synthesized in its unorganized tissue culture or callus. Most times, tissue differentiation is a requirement. Previous work on essential oils obtained from Apiaceae tissue culture indicated that some components of the essential oil like the phenylpropanoids can be synthesized in the callus but not the mono- nor the sesquiterpenes (Cardenas, 1993). Thus, the essence produced in tissue culture can only approximate the full scent of the plant depending partly on the degree of tissue differentiation attained.

"Camia", *Hedychium coronarium* J. König of the Zingiberaceae, carries short-lived, white, sweet scented flowers. A study on the tissue culture of camia flowers was attempted to check on any natural products it can synthesize and store. A growth medium previously proven to sustain alkaloid production in *Catharanthus roseus* (L.) G. Don floral tissue culture was used (Cardenas, 1983).

Materials and Methods

Floral tube of yet unopened camia flowers was used as explant for the experiment. Whole unopened flower buds were surface sterilized in 3% CaOCl aqueous solution for 5 mins. The floral tube was excised after three consecutive rinsing in sterile distilled water and cut into 1-mm sections. These were inoculated into two different growth media (Table 1). The first was "MS" Murashige and Skoog medium (Murashige and Skoog, 1962) and the second "mWB" was a modification of the Wood and Braun medium (Braun and Wood, 1962). The two media differed only in their macroelements. Their microelement and vitamin composition are similar following those recommended for MS medium. Both media were supplemented with 3 ppm NAA (naphthalene acetic acid) and 0.5 ppm Ki (kinetin), 3% sucrose and 0.2% Gelrite. The pH was adjusted to 5.8 prior to autoclaving.

The cultures were maintained at ambient room temperature of 28°C with 8-hr daylight provided by a west-facing window. Low diffused light, with the highest value at 5.20 $\mu molS^{-1} m^{-2}$ recorded in the early afternoon, was observed. Observation on the basic anatomy of the growing callus was made after 6 weeks of culture under the light microscope. The orange pigment produced by the callus was extracted with absolute methanol in the dark and the absorption spectra in visible light (380-780 nm) determined using Labomed® spectro dual split beam UV-VIS spectrophotometer, USA.

Table 1. Comparison of the MS and mWB growth media used in the experiment.

MS (*Physiol. Plant.* 18: 100) in mg/l:		mWB (*PNAS* 48: 1776) in mg/l:	
370	$MgSO_4$-$7H_2O$	$MgSO_4$-$7H_2O$	1,368
		Na_2SO_4	200
440	$CaCl_2$-$2H_2O$	$Ca(NO_3)_2$	288
170	KH_2PO_4	KCl	925
		NaH_2PO_4	316.5
1900	KNO_3	KNO_3	80
		$NaNO_3$	1,800
1650	NH_4NO_3	$(NH_4)_2SO_4$	790

Results and Discussion

The floral tube segments produced callus slowly in two months from inoculation with the 3ppm NAA and 0.5 ppm Ki supplements. Unexpectedly, though, the white explant on mWB changed to orange in color and this color was maintained in the callus that developed. Succeeding subcultures of the callus to media of the same composition proved that the pigment was, indeed, synthesized and sequestered in all cells of the tissue culture. Seldom is chlorophyll produced (Fig. 1). In contrast, MS-grown callus remained mostly unpigmented, typical of cultures from unpigmented explants. As the latter matured, roots were initiated (Fig. 2).

For the Zingiberaceae, the most studied pigments are the curcuminoids: curcumin, monodemethoxycurcumin and bisdemethoxycurcumin of the *Curcuma* species. These are cinnamoyl pigments produced and stored in the rhizome with recorded biological activities, particularly for curcumin (Wagner and Bladt, 1996). On the other hand, no pigment analysis for *H. coronarium* was encountered. Fruit set among the local populations elsewhere is apparently low, unlike the plants of *H. coronarium* at the Singapore Botanic Gardens that produced big orange fruits during the time of the 4th International Symposium on the Zingiberaceae in July 2006. The fruit of the local species populations in the Philippines, encountered only once by this researcher in October 2006, is small and hidden inside the floral bract.

The orange color of fruits is mostly attributed to carotenoids and there are over 600 natural carotenoids known, including those in algae, fungi and bacteria (Rodriguez-Amaya, 1999). It can not be discounted that the orange pigment(s) synthesized in camia callus is of the carotenoids.

Figure 1. *H. coronarium* callus from floral tube explant two months after inoculation on mWB medium supplemented with 3ppm NAA and 0.5 ppm Ki.

Figure 2. *H. coronarium* callus from floral tube explant two months after inoculation on MS medium supplemented with 3ppm NAA and 0.5 ppm Ki.

Anatomical observations of the callus showed some short trichomes that might be a carry over from the explant. Unorganized callus growth was evident. Compared with carrot and squash that are well studied for their carotenoids, the camia callus methanol extract exhibited a different TLC (thin layer chromatography) profile. Likewise, there are differences in the visible absorption spectra of the methanol extracts of the three plant species (Fig. 3).

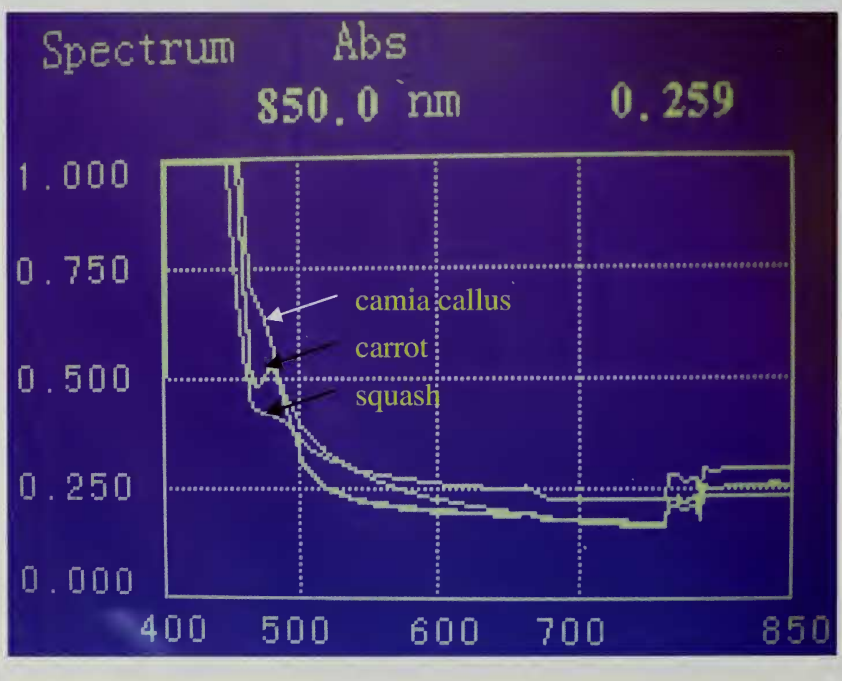

Figure 3. Absorption spectra of the methanol extracts of camia callus, squash and carrot.

The production of the pigment in camia callus might be due to the composition of the macroelements in the medium as this was the only difference between the media. [Succeeding camia floral tube cultures on mWB medium initiated and maintained in growth room of 16-hr light at 16.0 $\mu molS^{-1}m^{-2}$ provided by fluorescent lamps and in controlled temperature range of 17-24 °C also produced orange pigmented callus.] The possibility of osmotic value difference, however, was not discounted. An experiment using mannitol as osmolyticum will be pursued to check this factor.

The pigment in camia callus grown on mWB medium is likely mainly carotenoids, but this is yet to be ascertained. Lately, the researcher was able to secure a flowering and fruiting specimen of *H. philippinense*. The flowers of this epiphyte are yellow and the fruits are orange reaching 6 cm at maturity. In the absence of *H. coronarium* fruit, pigment of this species' fruit will be used as reference in the further analysis of the camia callus pigment. Modifications in the TLC procedure and in the protocol to obtain absorption spectra of the pigment will be pursued.

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